

novel genetically modified mouse line expressing the r/hM3Dq DREADD under the control of an MCK promotor allowed specific Gq activation by the design drug CNO. 5  $\mu$ M CNO (blood plasma concentration) induced massive cardiac arrhythmias (sinus arrest, AV-blocks, tachycardia and deformed QRS complexes) within minutes after injection only in r/hM3Dq expressing mice. Isolated hearts and experiments on ventricular and atrial myocytes supported such in-vivo findings. From these data we conclude that Gq-coupled signaling exerts genomic and non-genomic changes on the heart well exceeding the purely InsP3 dependent signaling and thus offers novel insights in cardiac rhythm generation and propagation in health and disease.

#### 657-Pos Board B437

##### The Large Scale Transcriptome Analysis of Mouse Sinoatrial Node (SAN) Yosuke Okamoto, Kirill V. Tarasov, Bruce D. Ziman, Joonho Lee, Edward G. Lakatta.

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Novel functional analyses and numerical models have led to a paradigm shift in sinoatrial nodal pacemaker cell biology. Recent reports of transcriptional profiles of SAN, however, have focused on a limited number of pre-selected transcripts, while the large-scale transcriptome profile of SAN pacemaker cells remains unknown. Using Illumina Ref8v2 bead chips we performed large-scale transcriptome analysis of sinoatrial node (SAN) and compared it to those of left and right atrium (LA and RA), left and right ventricle (LV and RV) and spontaneously beating cultured HL-1 cells.

Principal component analysis of transcriptomic relationship among samples revealed a distinct supraventricular grouping (LA, RA, SAN and HL-1), a ventricular grouping (LV, RV), a native heart tissue grouping (LA, RA, SAN, LV and RV) and the cultured cell line. Overexpressed genes specific to each native heart tissue (greater than 75th percentile for that group) were 3946 transcripts evenly expressed among its component tissues. The supraventricular group expression of 87 of these genes was specifically enriched in SAN; 95, in RA and 205 in LA; ventricular grouping embraced 2773 common transcripts, with 901 transcripts being enriched in LV and 142 in RV. Some findings in SAN compare to other heart tissues, for example, PDE1A, Tbx3, RGS4, ISL1, were highly overexpressed. The co-expression gene networks by the weighted gene co-expression analysis reveal that neuron projection, fatty acid metabolic process, neuron differentiation and response to endogenous stimulus pathways were highly represented in SAN.

In summary, this is the first large-scale transcriptomic analysis of cardiac pacemaker tissue and its comparison to other cardiac tissue. Novel gene markers for SAN were observed. The results provide valuable clues with respect to regeneration of SAN-like beating cells from pluripotent cell lines and to the design of biological pacemakers.

#### 658-Pos Board B438

##### A Simple Regulation of Cardiomyocyte Excitability

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Introduction: Recently, genome wide association studies (GWAS) identified LITAF (lipopolysaccharide-induced TNF factor) as one of the novel loci associated with prolongation of QT interval. Our goal is to investigate the role of LITAF in regulating cardiomyocyte excitability and action potential duration (APD). Methods: Optical mapping was performed in zebrafish hearts to determine  $\text{Ca}^{2+}$  transients and transmembrane (TM) potentials. Propagation velocities of depolarizing waves were determined. Action potential (AP) was digitally imaged in adenoviral infected neonatal rabbit cardiomyocytes (NRbCM). Live cell confocal calcium imaging was performed on adult rabbit ventricular myocytes (ARbCM) to determine intracellular  $\text{Ca}^{2+}$  handling. A current-voltage (I-V) relationship for the L-type calcium current ( $\text{I}_{\text{Ca,L}}$ ) was determined using whole cell patch clamping. To study the effect of LITAF on Cav1.2 channel expression, surface biotinylation and western blot assays were performed using tSA201 cells. Results: Our studies demonstrate that loss-of-function of LITAF in vivo (zebrafish) increases the  $\text{Ca}^{2+}$  transient amplitude (which primarily depends on calcium influx), slows atrial-ventricular conduction, and prolongs the APD. In contrast, in vitro gain-of-function experiments in rabbit CM established that overexpression of LITAF downregulates the total and surface pools of Cav1.2 channel polypeptides, decreases  $\text{Ca}^{2+}$  transients and L-type  $\text{Ca}^{2+}$  conductance, and shorten the APD. Studies in heterologous expression systems revealed that one of the auxiliary subunits of Cav1.2 channels, Cav $\beta$ 2b, is likely involved in LITAF-mediated

inhibition of recombinant Cav1.2 channels. Conclusion: LITAF regulates the expression and function of Cav1.2 channels, thereby determining APD and cardiac cell excitation.

#### 659-Pos Board B439

##### Mitochondrial Abnormalities in a Mouse CPVT Model with RyR2 Loss-Of-Function Mutation

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Mitochondria exchange matrix content in a unique and dynamic way by one of two mechanisms: a short-range direct interaction via closely spaced kissing junctions or long-range propagation of signal by the extrusion of extended nanotubular extensions (nanotunneling) (Huang et al 2013. Proc Natl Acad Sci U S A, 110(8): 2846-51). Both are normal, but infrequent features of cardiac myocytes. It is interesting to know whether this behavior may be affected by changes in  $\text{Ca}^{2+}$  homeostasis. We evaluated the cardiac ultrastructure in 7-month old heterozygous mice with a cardiac ryanodine receptor (RyR2) mutation (RyR2-A4860G) that depresses channel function and leads to Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) without hypertrophy (Zhao et al., submitted). The mutation is embryonic lethal in mice homozygous for the mutation. In ventricular myocytes of heterozygotes mice (RyR2-A4860G<sup>+/-</sup>) it has a dual effect on SR  $\text{Ca}^{2+}$  release: an initial reduction due to lower RyR2 activity, linked to random bursts due to SR  $\text{Ca}^{2+}$  overload. The most striking and only structural alteration in cardiac myocytes in the mutant left ventricle is a clear increase in the frequency of long thin nano-tunneling mitochondrial extensions. The frequency of nanotunneling was ~3 fold higher in mutant than in WT mice. Numerous small mitochondrial profiles are more frequent in mutant mice resulting in an average surface area of  $0.17 \pm 0.15 \mu\text{m}^2$  in mutant versus  $0.28 \pm 0.22 \mu\text{m}^2$  in WT ( $p < 0.00001$ ). This decrease is mostly due to sections through the thin tunneling extensions. The increased nanotunneling frequency would indicate an enhanced rate of long range intermitochondrial signal transfer. Interestingly, no activation of nanotunneling has been reported by EM in other CPVT models and/or in other alterations affecting proteins of CRU and  $\text{Ca}^{2+}$  homeostasis, making this a unique mitochondrial response.

#### 660-Pos Board B440

##### Wnt Signaling Selectively Inhibits Sodium Channels in Cardiac Myocytes Wenbin Liang, Eduardo Marbán.

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**Background:** Wnt signaling plays crucial roles in heart development but is normally suppressed postnatally. In arrhythmogenic conditions, such as cardiac hypertrophy and heart failure, Wnt signaling is reactivated. We explored the potential role of Wnt signaling in arrhythmogenic electrical remodeling by examining ion channel changes in postnatal cardiomyocytes with activated Wnt signaling.

**Methods:** Neonatal rat ventricular myocytes (NRVMs) were treated with either recombinant Wnt3a protein (0.1-1.0  $\mu\text{g/ml}$ ) or CHIR-99021 (CH, 3  $\mu\text{M}$ , a glycogen synthase kinase-3 $\beta$  inhibitor) to activate canonical Wnt signaling. Gene expression was assayed by real-time RT-PCR. Western blot and immunocytochemistry were used to investigate protein changes. Ionic currents were recorded using whole-cell patch-clamp.

**Results:** Treatment of NRVMs with either Wnt3a or CH caused a dose-dependent increase in Wnt target gene expression (Axin2 and Lef1), indicating activation of the Wnt/ $\beta$ -catenin pathway. Cardiac  $\text{Na}^+$  current ( $\text{I}_{\text{Na}}$ ) density was reduced ( $p < 0.01$ ,  $n > 10$ ) by Wnt3a ( $-20 \pm 4$  vs. control  $-59 \pm 7$  pA/pF, at  $-30$  mV) or CH ( $-22 \pm 5$  pA/pF), without changes in steady-state inactivation or repriming kinetics. Wnt3a and CH also produced dose-dependent reductions in the mRNA level of Scn5a (the cardiac  $\text{Na}^+$  channel  $\alpha$  subunit gene), as well as a 60% reduction in Nav1.5 protein level. Consistent with  $\text{I}_{\text{Na}}$  reduction, action potentials in Wnt3a-treated NRVMs had lower ( $p < 0.01$ ,  $n = 5$ ) upstroke amplitude ( $91 \pm 3$  vs. control  $137 \pm 2$  mV) and decreased maximum upstroke velocity ( $70 \pm 10$  vs. control  $163 \pm 15$  V/s). In contrast, inward rectifier  $\text{K}^+$  current and L-type  $\text{Ca}^{2+}$  current were not affected by Wnt3a treatment ( $n = 8$ ).

**Conclusions:** The Wnt/ $\beta$ -catenin pathway selectively suppresses  $\text{I}_{\text{Na}}$  expression in postnatal cardiomyocytes and may contribute to arrhythmogenic remodeling in heart failure. Thus, the Wnt/ $\beta$ -catenin pathway may be a novel therapeutic target for heart failure and cardiac arrhythmias.